

### Alkaline Phosphatase in the Mesentery and Epididymal Fat Pad of Normal and Hypertensive Rats

Alkaline phosphatase activity in blood vessels is most marked in the adventitia of arteries and capillary endothelium<sup>1-5</sup>. At a histochemical level the arterioles of rats with DOC hypertension show increased alkaline phosphatase in the adventitia<sup>6</sup>, and quantitative studies on the aortic wall of rats with hypertension due to experimental renal artery stenosis has shown increased activity of the enzyme<sup>7</sup>.

In the experiments presented here we have carried out a combined quantitative and histochemical investigation of alkaline phosphatase in samples of mesentery and epididymal fat pad from normal rats and rats with DOC hypertension. Mesentery was chosen as an example of a fatty tissue containing numerous small muscular arteries that show well marked changes in hypertension and epididymal fat pad as a fatty tissue containing few blood vessels.

**Materials and methods.** Sixteen albino male rats with an initial body weight of about 280 g were maintained on a 4% NaCl medium carbohydrate diet<sup>8</sup> and given free access to tap water. A right nephrectomy was performed on all the rats and 5 of these animals served as controls. The remaining 11 rats were injected intramuscularly each day with 3 mg 11-deoxycorticosterone (DOC) in sesame oil for 7 weeks. During this period 2 of the DOC-treated rats died and at necropsy their organs showed the classical changes of hypertension. At the end of the experiment the blood pressure of all the surviving rats was measured by direct cannulation of the aorta under light ether anaesthesia following which they were killed by exsanguination.

Tissue samples from mesentery and epididymal fat pad of about 500 mg were weighed and homogenized in chilled bicarbonate-saline, and the alkaline phosphatase activity of the homogenate was determined by a modification of the King-Armstrong method<sup>9</sup>. Adjacent samples of mesentery and fat pad were also taken for the histochemical localization of the enzyme by the Gomori method<sup>10</sup>, and blocks from the heart, left kidney, mesentery and fat pad were processed for routine histological examination.

**Results.** See accompanying Table. The rats treated with DOC became hypertensive and their heart weight and the weight of the remaining kidney was greater in proportion to body weight than in the control animals. Microscopically the heart, kidney and mesentery showed the characteristic proliferative and degenerative lesions in the small arteries and arterioles that have frequently been described in this condition<sup>8,11</sup>. Sections from the epididymal fat pads contained few vessels above capillary size and these rarely had hypertensive changes.

The quantitative determinations of alkaline phosphatase gave low values for the homogenates from the epididymal fat pads and the mesenteries of the control animals. In the hypertensive rats the alkaline phosphatase of the fat pads was not significantly different to that of the controls ( $P > 0.5$ ), but the alkaline phosphatase activity of the mesenteric homogenates was significantly increased, being about five times above the control level ( $P < 0.01$ ).

The sections stained histochemically for alkaline phosphatase showed only slight activity in the fat pads and mesenteries from the control rats. The reaction was practically confined to the adventitia of small arteries, where it had a rather patchy distribution. There was a moderately increased alkaline phosphatase activity in the fat-

pad sections from only 3 of the 9 hypertensive rats, but the corresponding mesenteries showed a marked increase in every case. The small mesenteric arteries and the arterioles frequently showed a dense histochemical reaction throughout the adventitia. Where there was a periadventitial cellular infiltrate or an infiltrate extending through the adventitia into the media to produce an arteritic type of lesion, the cells of this infiltrate showed marked activity as well. The only other foci of alkaline phosphatase activity in the sections were occasional isolated cells, probably histiocytes. These findings suggest that the increased activity found quantitatively in the mesenteric homogenates from the DOC-treated rats was due almost entirely to the increased activity present in the walls of the small hypertensive arteries and arterioles.

Alkaline phosphatase activity in mesentery and epididymal fat pad of DOC hypertensive and control male rats

	Hypertensive	Control
No. of rats	9	5
Body weight, g		
Initial	283 ± 3.2	286 ± 2.6
Final	356 ± 9.7	430 ± 6.8
Blood pressure, mm Hg	190 ± 6.0	139 ± 1.9
Organ weight, mg/100 g		
body weight:		
Heart	443 ± 11.0	312 ± 3.6
Left kidney	723 ± 22.0	534 ± 15.9
Alkalinephosphatase,		
King-Armstrong units/g:		
Mesentery	2.01 ± 0.08 <sup>a</sup>	0.39 ± 0.04 <sup>a</sup>
Epididymal fat pad	0.23 ± 0.06 <sup>b</sup>	0.25 ± 0.09 <sup>b</sup>

Results are expressed as means ± standard errors. <sup>a</sup>  $P < 0.01$ . <sup>b</sup>  $P > 0.50$ .

**Zusammenfassung.** In der vorliegenden Arbeit wird die Phosphataseaktivität im Mesenterium und Fettpolster des Nebenhodens normotensiver und hypertensiver Ratten durch chemische Quantitätsbestimmung und histochemische Lokalisation bestimmt.

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<sup>1</sup> G. GOMORI, Proc. Soc. exp. Biol. Med. 42, 23 (1939).

<sup>2</sup> H. TAKAMATSU, Trans. Soc. Path. Jap. 29, 492 (1939).

<sup>3</sup> G. GOMORI, J. cell. comp. Physiol. 17, 71 (1941).

<sup>4</sup> E. A. KABAT and J. FURTH, Am. J. Path. 17, 303 (1941).

<sup>5</sup> G. BOURNE, Quart. J. exp. Physiol. 32, 1 (1943).

<sup>6</sup> D. L. GARDNER, Quart. J. exp. Physiol. 48, 156 (1963).

<sup>7</sup> W. ALBRECHT, Z. Naturforsch. [B] 18, 871 (1963).

<sup>8</sup> W. A. J. CRANE, G. F. WILGRAM, and D. J. INGLE, Scot. med. J. 5, 437 (1960).

<sup>9</sup> E. J. KING and A. R. ARMSTRONG, Canad. med. Ass. J. 31, 376 (1934).

<sup>10</sup> A. G. E. PEARSE, *Histochemistry: Theoretical and Applied* (J. and A. Churchill, London 1960), p. 869.

<sup>11</sup> H. SELYE, C. E. HALL, and E. M. ROWLEY, Canad. med. Ass. J. 49, 88 (1943).